Multifunctional Bone-Targeting Peptides for Inhibition of the RANK-TRAF6 Interaction

P2.373

https://doi.org/10.17952/38EPS.2026.P373

Sofia A. Martins[1], Cátia Rosa[1], Marco Cavaco[2], Rita Melo[1], David Andreu[3], Javier Valle[3], Vera Neves[2], Miguel A. R. B. Castanho[2], Sandra Casimiro[2], João D.G. Correia[1,4]

[1] Center for Nuclear Sciences and Technologies, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, ao km 139,7; 2695-066 Bobadela LRS, Portugal;

[2] Gulbenkian Institute of Molecular Medicine, Professor Egas Moniz 1649-028 Lisboa, Portugal;

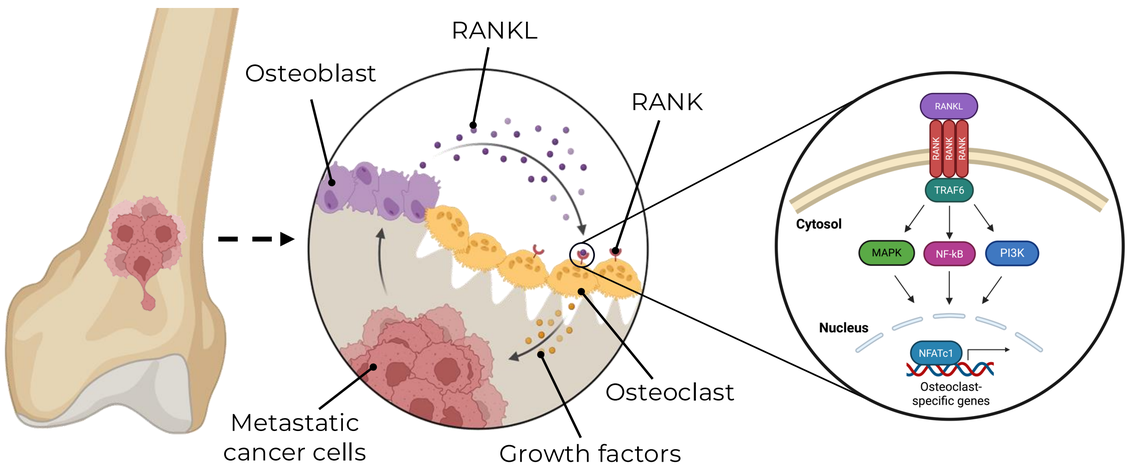
[3] Department of Medicine and Life Sciences, Pompeu Fabra University, Dr. Aiguader 88, Barcelona Biomedical Research Park, 08003 Barcelona, Spain;

[4] Department of Nuclear Sciences and Engineering, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, ao km 139,7; 2695-066 Bobadela LRS, Portugal

1. Background

Bone metastases remain a major cause of **cancer-related mortality**[1].

The bone undergoes **constant remodelling** during an individual's lifetime. This **remodelling** is **disrupted** in **metastasis**[1].



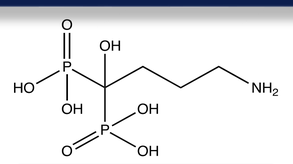
The vicious cycle of bone metastasis and its signalling pathways

Cancer cells induce **osteoblasts** to secrete receptor activator of nuclear factor kappa B ligand (**RANKL**), which binds to its receptor, **RANK**, in **osteoclasts**[1].

Through tumour necrosis factor receptor associated factor 6 (**TRAF6**), this interaction increases **osteoclast activity**, promotes **bone resorption** and **secretion of growth factors**[1].

2. Aim

Develop **multifunctional peptide conjugates** that **disrupt** the **RANK/TRAF6** axis and thus **hinder osteoclastogenesis** and **metastatic potential**



\+ Fragment A +

① **Bone-targeting unit**

A bisphosphonate **(alendronate)** with **high affinity** for **bone mineral**[2]

② **Cathepsin K-sensitive linkers**

Cathepsin K is an abundant **protease** in the **bone microenvironment**[3]

Fragment B + Fragment C

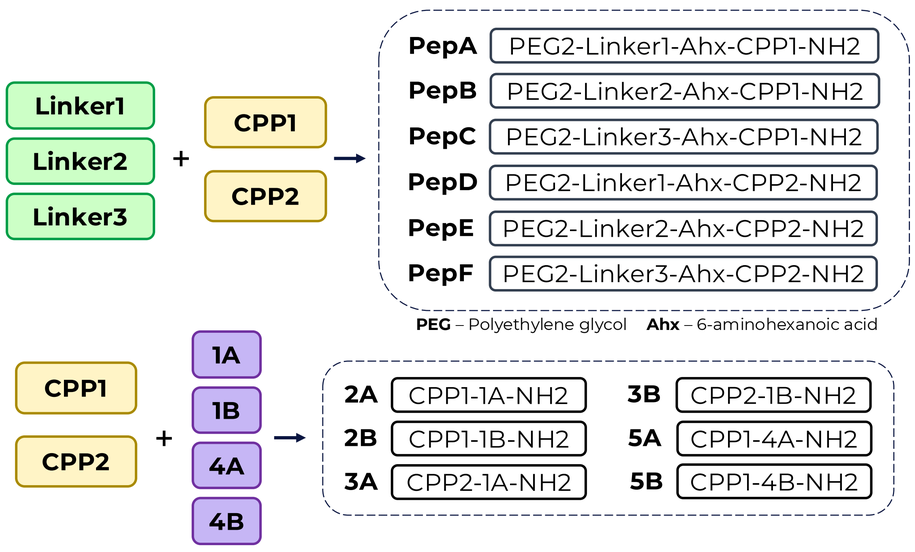
③ **Cell-penetrating peptides (CPPs)**

Peptide sequences able to promote **cell entry** of different cargoes[4]

④ **RANK-derived sequences**

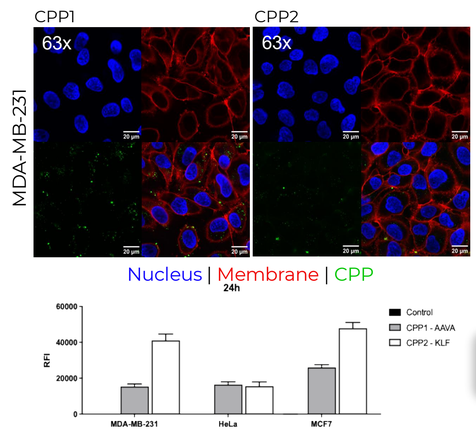
Bind to **TRAF6** and **disrupt** the **RANK/TRAF6 interface**[5]

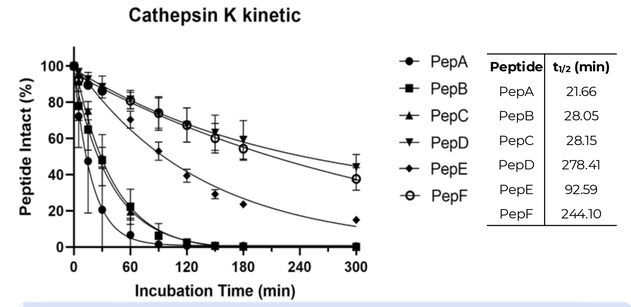
3. Approach



4. Results

Cell Internalization and Cathepsin K Cleavage

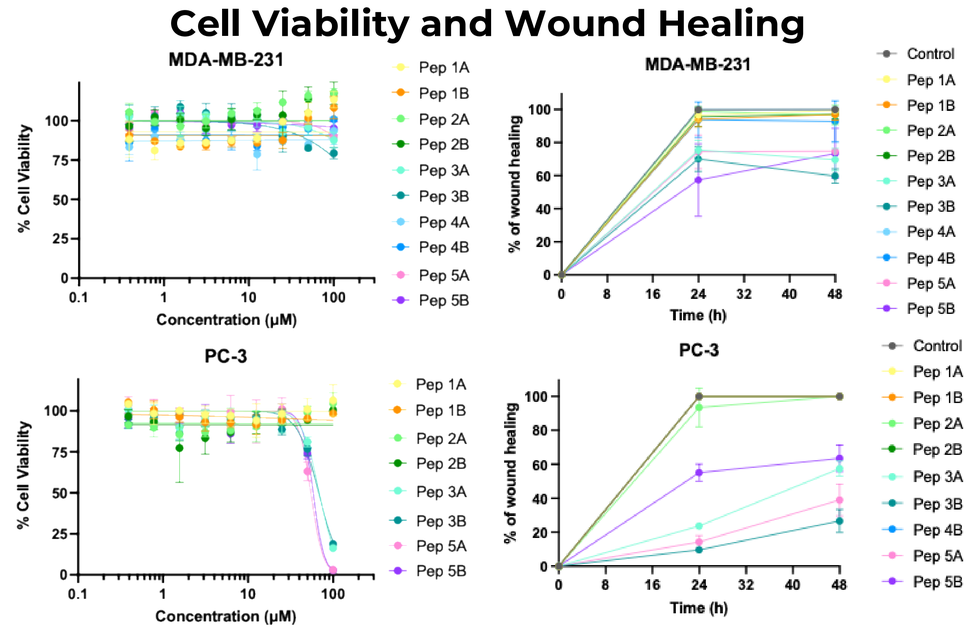




Peptide	$t_{1/2}$ (min)
PepA	21.66
PepB	28.05
PepC	28.15
PepD	278.41
PepE	92.59
PepF	244.10

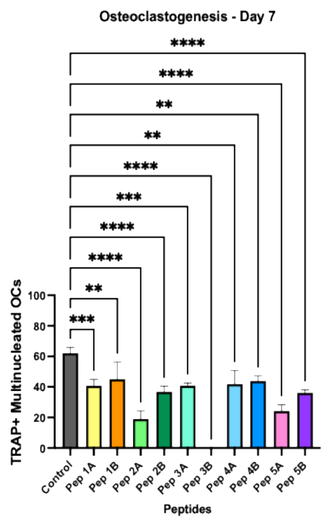
Both CPPs are **internalized; peptides A, B and C** show **faster cleavage kinetics** than D, E and F.

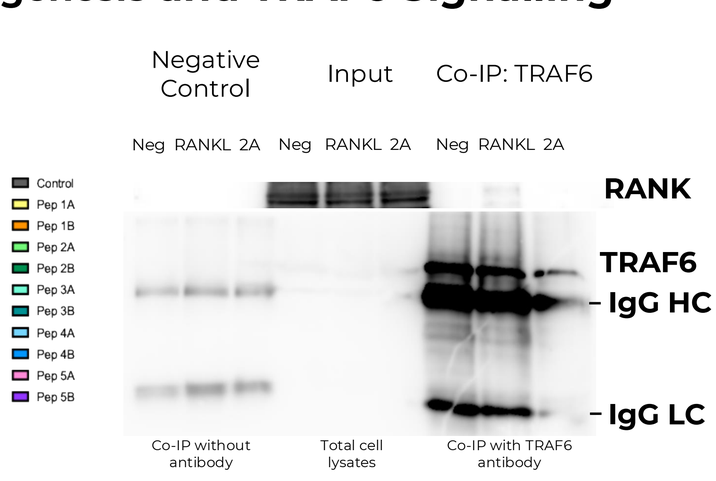
Cell Viability and Wound Healing

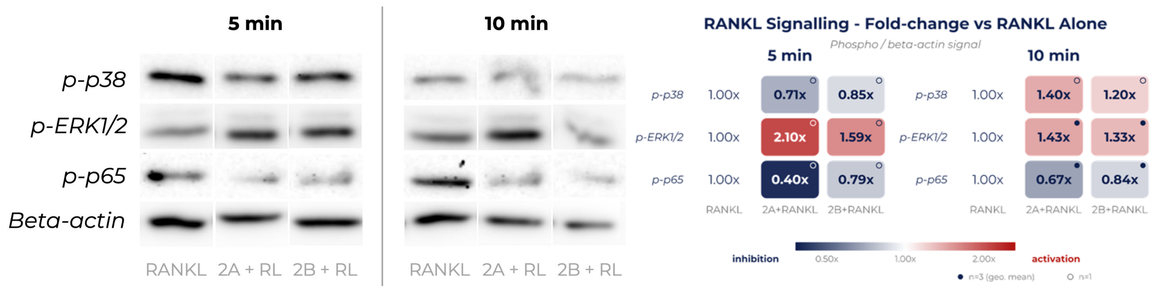


Only **3A, 3B, 5A and 5B** show **slight cytotoxicity** and also **impair wound healing** in **prostate cancer cells** – likely **reflecting cytotoxicity, not an anti-migratory effect**.

Osteoclastogenesis and TRAF6 Signalling







2A and 5A show the **strongest inhibition of osteoclastogenesis**; **3B** is **cytotoxic** to **monocytes. 2A disrupts RANK/TRAF6,** and seems to **modulate downstream signalling**.

5. Conclusions

- **Both CPPs display internalization** and **cleavage kinetics** seems to strongly depend on the **CPP sequence** rather than on the **type of cathepsin K-sensitive linker** – supporting **CPP selection** as **key for cargo release**.
- Peptides **3A, 3B, 5A and 5B** exhibit **slight cytotoxicity** and **impair wound healing**, but **re-evaluation** at **sub-cytotoxic concentrations** is **warranted**.
- Peptides **2A** and **5A** show the **highest inhibitory effect in osteoclastogenesis,** highlighting their **potential** as the **most promising anti-resorptive candidates.**
- Peptide **2A** can **disrupt the RANK/TRAF6 interface; both 2A and 2B** appear to **modulate downstream signalling**.
- Taken together, these results support the **selection of Linker 1 and 2A** as the **lead candidates** for the **development** of the **full peptide**.

REFERENCES

1. Kottmann, V. *et al.* Biochim. Biophys. Acta – Rev. Cancer. 189551 (2026).
2. Bruschi, A. *et al.* Cancer Med. 14(9), e70901 (2025).
3. Dey, G. *et al.* Jacs Au. 5(3), 1104 (2025).
4. Jankowski, A. M. *et al.* Nanoscale. 17(35), 20006-20019 (2025).
5. Anzai, M. *et al.* Commun. Biol. 8(1), 643 (2025).

ACKNOWLEDGEMENTS

This work was supported by FCT through PTDC/QUI-OUT/3854/2021, UI/BD/154474/2022 and UID/Multi/04349/2020.